

We claim:

1. A method for forming an isolated ribonucleoprotein complex comprising:
providing an RNA affinity substrate comprising a ribonucleoprotein assembly sequence
and an affinity tag;
contacting the RNA affinity substrate with a protein mixture so as to permit the formation
of a ribonucleoprotein complex on said ribonucleoprotein assembly sequence;
subjecting said ribonucleoprotein complex to chromatographic separation; and
subjecting said ribonucleoprotein complex to affinity selection, wherein the affinity tag
binds to an affinity matrix;
thereby forming an isolated ribonucleoprotein complex.
2. The method of claim 1, further comprising eluting said ribonucleoprotein complex from
said affinity matrix by disrupting the interaction of the affinity tag with the affinity
matrix.
3. The method of claim 1, wherein said ribonucleoprotein complex is selected from the
group consisting of a spliceosomal complex, an hnRNP complex, an mRNA export
complex, an mRNA localization complex, an RNA editing complex, and an intron
complex.
4. The method of claim 3, wherein the ribonucleoprotein complex is a spliceosomal
complex selected from the group consisting of an E complex, an A complex, a B complex
and a C complex.
5. The method of claim 3, the ribonucleoprotein complex is an H complex.
6. The method of claim 1, wherein the ribonucleoprotein assembly sequence is selected
from the group consisting of a pre-mRNA sequence, a 5' splice site, a 3' splice site, and
an intronless RNA.

7. The method of claim 1, wherein the affinity tag binds to an affinity matrix through the intermediate of a fusion protein comprising a polypeptide binding specifically to the affinity tag and a polypeptide binding specifically to the affinity matrix.

5 8. The method of claim 7, wherein the affinity tag comprises at least one MS2 or R17 coat protein recognition site and the polypeptide binding specifically to the affinity tag is an MS2 or R17 coat protein or portion thereof sufficient for binding to the MS2 or R17 coat protein recognition site.

10 9. The method of claim 7, wherein the polypeptide binding specifically to the affinity matrix is selected from the group consisting of a maltose binding protein; a 6x His peptide; glutathione S transferase; or portion thereof sufficient to bind specifically to an affinity matrix.

15 10. The method of claim 9, wherein the polypeptide binding specifically to the affinity matrix is a maltose binding protein or portion thereof sufficient to bind to amylose, the affinity matrix is an amylose matrix, and the ribonucleoprotein complex is eluted from the amylose matrix with maltose or a maltose analog.

20 11. The method of claim 7, comprising contacting the RNA affinity substrate with the fusion protein, such that the fusion protein binds specifically to the affinity tag prior to contacting the RNA affinity substrate with the protein mixture.

25 12. The method of claim 1, wherein the protein mixture is a eukaryotic cell nuclear extract or a subfraction thereof.

13. The method of claim 1, wherein the chromatographic separation is a gel filtration.

30 14. The method of claim 1, wherein the affinity selection is performed in a low ionic strength buffer.

15. The method of claim 14, wherein the low ionic strength buffer comprises a final salt concentration of less than about 100 mM.

16. The method of claim 1 for isolating a spliceosome comprising:

providing an RNA affinity substrate comprising a pre-mRNA sequence and an MS2 coat protein recognition site;

contacting the RNA affinity substrate with a fusion protein comprising (i) an MS2 coat protein or portion thereof sufficient to bind specifically to the MS2 coat protein recognition site and (ii) a polypeptide binding specifically to a ligand, such that the fusion protein binds to RNA affinity substrate;

contacting the RNA affinity substrate with a eukaryotic cell nuclear extract so as to permit the formation of a spliceosome mRNA complex;

subjecting the spliceosome mRNA complex to chromatographic separation; and

subjecting the spliceosome mRNA complex to affinity selection on an affinity matrix comprising the ligand,

thereby isolating a spliceosome.

17. The method of claim 16, wherein the RNA affinity substrate comprises at least two MS2 coat protein recognition sites.

18. The method of claim 16, wherein the polypeptide binding specifically to a ligand is selected from the group consisting of a maltose binding protein; a 6x His peptide; glutathione S transferase; or portion thereof sufficient to bind specifically to the ligand.

19. The method of claim 18, wherein the polypeptide binding specifically to a ligand is a maltose binding protein or portion thereof sufficient to bind to amylose; wherein the affinity selection comprises binding of the spliceosome mRNA complex on an amylose matrix and eluting the ribonucleoprotein complex from the amylose matrix with maltose or a maltose analog.

20. An isolated spliceosome preparation, isolated by the method of claim 16.

21. The isolated spliceosome preparation of claim 20, wherein more than about 10% of the pre-mRNA sequences associated with said isolated spliceosome complexes can be chased into a completely spliced mRNA in a splicing reaction.

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22. The isolated spliceosome preparation of claim 20, comprising a quantitative amount of 17S U2 U2 small nuclear ribonucleoprotein (snRNP).

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23. The isolated spliceosome preparation of claim 20, comprising a quantitative amount of an SP3a polypeptide.

24. The isolated spliceosome preparation of claim 20, comprising at least 90% of the proteins listed in Tables 1 and 2.

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25. The isolated spliceosome preparation of claim 23, wherein said spliceosome preparation is an E complex spliceosome preparation.

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26. The isolated spliceosome preparation of claim 20, wherein said spliceosome preparation is an A complex spliceosome preparation.

27. A ribonucleic acid comprising a ribonucleoprotein complex binding site and at least one phage coat protein recognition site.

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28. The ribonucleic acid of claim 27, wherein the ribonucleoprotein complex binding site is a spliceosome binding site and at least one phage coat protein binding site is an MS2 or R17 coat protein recognition site.

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29. The ribonucleic acid of claim 27, wherein the spliceosome binding site is an adenovirus major late pre-mRNA or a fushi tarazu pre-mRNA.

30. A nucleic acid encoding the ribonucleic acid of claim 27.

31. The nucleic acid of claim 29, operably linked to an RNA promoter capable of transcribing the nucleic acid.

32. A diagnostic assay for determining whether a subject has abnormal ribonucleoprotein complexes, comprising:

obtaining a sample of cells from a subject;

purifying ribonucleoprotein complexes from the cells of the subject according to claim 1;

and

determining the presence in the purified ribonucleoprotein complexes of one or more proteins,

wherein a difference in the amount of one or more proteins in the ribonucleoprotein complexes of the subject relative to its amount in a corresponding normal ribonucleoprotein complex indicates that the subject has abnormal ribonucleoprotein complexes.

33. The diagnostic assay of claim 33, wherein ribonucleoprotein complexes are spliceosome complexes.

34. A diagnostic assay for determining whether a subject has abnormal spliceosome complexes, comprising:

obtaining a sample of cells from a subject; and

purifying spliceosome complexes from the cells of the subject according to claim 16;

determining whether the pre-mRNA sequence was spliced during the purification,

wherein splicing of the pre-mRNA sequence indicates that the spliceosome complexes of the subject are functional, whereas the absence of splicing or the pre-mRNA indicates that the spliceosome complexes of the subject are not functional, thereby indicating that the subject has abnormal spliceosome complexes.

35. A diagnostic kit comprising at least two elements selected from the group consisting of an RNA affinity substrate; a fusion protein comprising an affinity tag binding polypeptide

and a ligand binding polypeptide; a chromatographic separation reagent; and an affinity purification reagent.

36. A method for treating a subject having a disorder associated with abnormal ribonucleoprotein complexes, comprising
- 5 obtaining a sample of cells from a subject;
- purifying ribonucleoprotein complexes from the cells of the subject according to claim 1;
- determining the presence in the purified ribonucleoprotein complexes of one or more proteins; and
- 10 normalizing the amount of ribonucleoproteins in the subject,
- to thereby treat the subject having a disorder associated with abnormal ribonucleoproteins complexes.